Interactions of AtRGL1, a Negative Regulator of Gibberellic Acid Signalling

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Abstract

Arabidopsis thaliana AtRGL1 (repressor of *ga1-3* like-1) is a negative regulator of the signal transduction pathway of the plant hormone gibberellin. AtRGL1 belongs to the DELLA subfamily within the GRAS family of plant regulatory proteins. There are four other DELLA proteins, including AtRGA (repressor of *ga1-3*) and AtRGL2, encoded by the *A. thaliana* genome. Previous studies provided evidence that the DELLA proteins are nuclear localised and are functionally divided into N- and C-terminal domains. The N-terminal domain perceives the gibberellin signal, while the C-terminal domain functions as a negative regulator of transcription and also as a possible dimerisation domain. Previous studies have also shown that *AtRGA*, *AtRGL1*, and *AtRGL2* function together in the regulation of the development of the inflorescence and that *AtRGL1* is primarily expressed in this tissue.

To investigate how DELLA proteins function in gibberellin signalling, I sought plant proteins that interact with AtRGL1. Two proteins, p24 (24 kDa) and p64 (64 kDa), were isolated from wild-type plant nuclear extracts by affinity to the N-terminal 121 amino acid residues of AtRGL1. The identity of these two proteins remains to be established. To investigate the interactions of the C-terminal domain of AtRGL1 an anti-AtRGL1 polyclonal antiserum was developed for co-immunoprecipitation experiments. However, AtRGL1 was not detectable in plant nuclear extracts from the inflorescence of wild-type plants, precluding this approach.

The possibility of DELLA protein dimerisation was also investigated using AtRGA, AtRGL1, and AtRGL2 in yeast 2-hybrid experiments. Yeast 2-hybrid protein interaction results suggest that AtRGA, AtRGL1, and AtRGL2 do not form homo- or hetero-dimers. Complexities encountered with this approach could make these results invalid, so these interactions require further investigation.

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Abbreviations

3-AT	3-amino-1,2,4-trazole
ABA	Abscisic acid
AD	GAL4 activation domain
AMP	Ampicillin
$AmSO_4$	Ammonium sulfate
AP	Alkaline phosphatase
AtASK1	*A. thaliana* Arabidopsis Skp like-1
AtGAI	*A. thaliana* Gibberellic acid insensitive
AtGAMYB33	*A. thaliana* Gibberellic acid induced MYB-33
AtGAMYB65	*A. thaliana* Gibberellic acid induced MYB-65
AtGCR1	*A. thaliana* G-protein coupled receptor-1
AtGPA	*A. thaliana* G-protein α subunit
AtHDA19	*A. thaliana* Histone deacetylase-19
AtLFY	*A. thaliana* Leafy
AtLRP	*A. thaliana* Lateral root primordium
AtPKL	*A. thaliana* Pickle
AtrgaΔ17	*A. thaliana* Repressor of *ga1-3*, deletion of 17 amino acid DELLA motif
AtRGL1	*A. thaliana* Repressor of *ga1-3*-1
Atrgl1Δ17	*A. thaliana* Repressor of *ga1-3*-1, deletion of 17 amino acid DELLA motif
AtRGL2	*A. thaliana* Repressor of *ga1-3*-2
AtRGL3	*A. thaliana* Repressor of *ga1-3*-3
AtSHI	*A. thaliana* Short internodes
AtSLY1	*A. thaliana* Sleepy-1
AtSLY2	*A. thaliana* Sleepy-2
AtSNY	*A. thaliana* Sneezy (AtSLY2)
AtSPY	*A. thaliana* Spindly
BD	GAL4 DNA binding domain
BnSCL1	*B. napus* Scarecrow like-1
BSA	Bovine serum albumin
cDNA	complementary deoxyribonucleic acid
cGMP	Cyclic guanosine monophosphate
c-Myc	Mammalian c-Myc oncogene epitope

Co-IP	Co-immunoprecipitation
Col-0	Columbia-0
DAPI	4',6-Diamidino-2-phenylindole
DEAE	Diethylaminoethyl
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTT	1,4-Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
GA	Gibberellic acid
GAL4	GAL4 transcription factor
GARE	Gibberellic acid responsive element
GFP	Green Fluorescent Protein
HA	Influenza hemagglutin epitope
HDAC	Histone deacetylase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGP	Heterotrimeric G-protein
HRP	Horseradish peroxidase
HvCDPK1	*H. vulgare* Calcium dependent protein kinase-1
HvGAMYB	*H. vulgare* Gibberellic acid induced MYB
HvHSIMYB	*H. vulgare* Spindly interacting MYB
HvHSINAC	*H. vulgare* Spindly interacting NAC
HvSAD	*H. vulgare* Sad
HvSLN1	*H. vulgare* Slender-1
HvSPY	*H. vulgare* Spindly
IgG	Immunoglobulin G
Imidazole	1,3-Diaza-2,4-cyclopentadiene
IPTG	Isopropylthio–β-D-galactoside
KAN	Kanamycin
Ler-0	Landsberg *erecta*-0
MALDI TOF	Matrix assisted laser desorption ionisation time of flight
MBP	Maltose-binding protein
miR159	Micro ribonucleic acid 159
miRNA	Micro ribonucleic acid
mRNA	Messenger ribonucleic acid

MWCO	Molecular weight cut-off
NHS	*N*-hydroxysuccinimide
NLS	Nuclear localisation signal
O-GlcNAc	*O*-linked *N*-acetyl glucosamine
ONPG	*O*-Nitrophenol-galactoside
OsD1	*O. sativa* Dwarf-1
OsDOF3	*O. sativa* DNA binding with one finger-3
OsGID2	*O. sativa* Gibberellic acid insensitive dwarf-2
OsSLR1	*O. sativa* Slender rice-1
p24	24 kDa AtRGL1-interacting protein
p53	Murine p53
p64	64 kDa AtRGL1-interacting protein
PAGE	Poly-acrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Poly-ethylene glycol
PMSF	Phenylmethylsulphonylfluoride
SCF	Skp-Cullin-F-box
SD	Synthetic dropout
SDS	Sodium dodecyl sulfate
StPHOR1	*S. tuberosum* Photoperiod-responsive-1
T antigen	SV40 large T antigen
TBS	Tris-buffered saline
TBST	Tris-buffered saline Tween-20
Tris	Tris (hydroxymethyl) aminomethane
TrxA	Thyrodoxin domain A
UV	Ultra violet

Note on Nomenclature

Gene names and symbols are written in *italics*, and proteins are in standard text. Wild-type genes and products are in uppercase, mutants are in lower case. For plant species, the two letters preceding a gene/protein refer to the species. At, *Arabidopsis thaliana*. St, *Solanum tuberosum*. Hv, *Hordeum vulgare*. Os, *Orysa sativa*. Bn, *Brassica napus*.